BIOAG PROJECT PROGRESS OR FINAL REPORT TEMPLATE

TITLE: Bacterial and Fungal Symbioses and their Role in Increasing Plant Growth and Productivity

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ABSTRACT: This research addresses biologically intensive and organic approaches to improving plant nutrition, growth and productivity. Naturally occurring beneficial soil microbes are known to increase the acquisition of nutrients by plants. Controlling plant-microbe interactions under diverse environmental conditions should help in maximizing crop production. This should also help the industry to decrease its dependence on chemical fertilizers. Our lab has made exciting progress during the past year and a manuscript has been accepted for publication pending revision in The Plant Journal, which is an internationally recognized publication. Our goal has been to generate the necessary preliminary results to compete for larger grants from federal funding agencies.

PROJECT DESCRIPTION: A better understanding of bacterial and fungal symbioses could lead to increased crop productivity. The nutrient status of the soil (especially nitrogen and phosphorus) has a direct effect on microbial colonization. We have focused our research on both bacterial and fungal symbioses, and a manuscript describing our results is in the final stages of being published in The Plant Journal. The underlying hypothesis in our research is that there is an inverse relationship between nutrient status of the soil and bacterial and fungal colonization. For example, in the case of mycorrhizal symbiosis, this hypothesis indicates that if the soil has high phosphorus content, the fungal colonization decreases. Hence, it is believed that if the soil has high phosphorus, there may not be a detectable benefit from inoculating plants with mycorrhizal fungi.

To understand the control mechanism, we have focused our attention on the most commonly used species for this type of study, *Glomerales* (*Glomus* spp.). Our ultimate goal is to expand our research into different species of fungi that are involved in symbiosis in different geographic locations. Through discussions with Dr. Mark Mazzola and others, it is clear that there is a diversity of mycorrhizal species that are detected under different cropping systems. The use of *Glomus* is a starting point to get this project off the ground since it is commonly used by scientists as a model organism. Otherwise, one has to isolate various fungi that are unique to a particular location and then study their effects on different crops grown in that region. This requires larger funding and that is one of our future goals.

Our specific goal has been to generate preliminary data to compete for funding at the national level. With this in mind, we have focused on calcium and calcium/calmodulin-dependent protein kinase (CCaMK), which plays a critical role in the signaling pathway that establishes root nodule symbiosis (RNS) and arbuscular mycorrhizal symbiosis (AMS). A brief summary of our recent findings is presented below. Calcium-dependent phosphorylation is a central "switch" in the regulation of CCaMK and this has been shown to promote calmodulin binding, a key protein involved in symbioses. Our pending publication in The Plant Journal describes a novel regulatory mechanism of *Medicago truncatula* CCaMK (MtCCaMK) through the autophosphorylation of S344 in the calmodulin-binding/autoinhibitory domain. The phospho-ablative mutation S344A did not have significant impact on its kinase activities and supports RNS and AMS, indicating that phosphorylation at this position is not required for the establishment of symbioses. The phospho-mimic mutation S344D has drastically reduced calmodulin-stimulated substrate phosphorylation, and this coincides with a drastically compromised interaction with calmodulin and its interacting partner protein, IPD3. Functional complementation tests revealed that the S344D mutation completely blocked root nodule symbiosis and drastically reduced the mycorrhizal symbiosis. Furthermore, S344D was shown to suppress the spontaneous nodulation associated with a gain-of-function mutant of MtCCaMK (T271A), revealing that phosphorylation at S344 of MtCCaMK is adequate for shutting down its activity. These results reveal a novel mechanism of CCaMK which enables it to “turn off” its function through autophosphorylation. These findings have laid a solid foundation and should help us to approach funding agencies for further support to carry out both the basic and applied research in this area.

OUTPUTS

- Work Completed: We documented how to “turn on” and “turn off” both bacterial and fungal symbioses. We also have a better understanding of the conditions under which the microbes perform at the highest levels.
- Publications, Handouts, Other Text & Web Products: A manuscript was submitted to The Plant Journal which describes the above results. We have received word that the manuscript will be accepted for publication pending revision. This revision is now complete and we expect the manuscript to appear in print in the near future.
- Outreach & Education Activities: One graduate student has completed his PhD degree and a new one will be arriving in July 2013 and will continue this research.

IMPACTS

- Short-Term: Our results have provided the necessary baseline for practical use of beneficial microbes.
- Intermediate-Term: This study should provide the necessary foundation to promote sustainable agriculture and help in attracting federal grant support.
- Long-Term: Our results should provide both economic and environmental benefits to the state.

ADDITIONAL FUNDING APPLIED FOR / SECURED: Proposals to federal granting agencies will be submitted later this year.

GRADUATE STUDENTS FUNDED: We are pleased that WSU will be supporting our new PhD student for one year. This student will continue this research starting on July 1, 2013.

RECOMMENDATIONS FOR FUTURE RESEARCH: We are convinced that the use of naturally occurring beneficial microbes should be an integral part of any sustainable agriculture/organic farming initiative.